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(71) Applicant: **ABBOTT LABORATORIES** [US/US]; D377 AP6D, 100 Abbott Park Road, Abbott Park, IL 60064-6050 (US).

(72) Inventors: **BLANCHARD, Melanie, A.**; 18177 Meander Drive, Grayslake, IL 60030 (US). **MERSHIMER, Patricia, L.**; 1316 Elmwood Avenue, Wilmette, IL 60091 (US). **STELMACH, Honorate**; 36 East Atteridge Road, Lake Forest, IL 60045 (US). **TONER, Scott, E.**; 52 East White Barn Road, Vernon Hills, IL 60061 (US).

(74) Agents: **STEELE, Gregory, W.** et al.; Abbott Laboratories, D377 AP6D/2, 100 Abbott Park Road, Abbott Park, IL 60064-6050 (US).

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(54) Title: USE OF VITAMIN D_2 COMPOUNDS FOR ALOPECIA

(57) Abstract: The present invention discloses the use of vitamin D_2 compounds, particularly 1 α,25-dihydroxy-19-nor-vitamin D_2, for the prevention or treatment of chemotherapy-induced alopecia.

USE OF VITAMIN D_2 COMPOUNDS FOR ALOPECIA

Technical Field

The invention relates to a method for preventing and treating the condition of hair loss, known as alopecia. The invention more particularly relates to preventing and treating alopecia induced by the administration of chemotherapeutic agents. Active agents for use in the method of the invention are vitamin D_2 compounds, analogs, derivatives, or active metabolites thereof.

Background of the Invention

The condition of hair loss or baldness occurs as a side effect of the chemical therapy often necessary to treat various kinds of cancer. This side effect, commonly referred to as chemotherapy-induced alopecia, can have a devastating effect on the appearance of the patient. One fortunate aspect of alopecia is that the condition has an insignificant effect on the successful prognosis of cancer patients. Nonetheless, patients receiving chemotherapy often regard alopecia as a more important side-effect than other adverse reactions, including even vomiting. Tierney, A., et al. "Hair Loss due to Cytotoxic Chemotherapy: A Prospective Descriptive Study," *Br. J. Cancer*, **62**: 527-528 (1990).

Some of the most widely-used chemotherapeutic agents have potent alopecia-inducing effect. For example, adriamycin (ADM), cytoxan (CTX), etoposide (VP-16) and paclitaxel, are all clinically available chemotherapeutic agents that can induce alopecia. Chemotherapeutic agents are often used in combination, which intensifies their alopecia effect. The chemotherapeutic agent CAF (CTX + ADM + 5-fluorouracil (5-FU)), however, is typically administered to patients for treatment of metastatic breast cancer. Patients treated under the CAF regimen typically lose 50% of their hair within 4-5 weeks after the first cycle of treatment. After a second cycle of treatment, more than 90% of patients become totally alopecic.

At present, there are no clinically available preventative measures or remedies for alopecia. Several approaches have been explored for diminishing or treating alopecia in a clinical setting. However, these methods are beset with complications and limitations.

In one method, a mechanical apparatus, called a scalp tourniquet, is applied to the patient's head just before chemotherapy to reduce blood flow to the blood vessels in the scalp. The reduced blood flow is intended to limit the amount of drug reaching the hair follicle. One limitation is that the scalp tourniquet technique is inconsistently applied, which produces variability in the therapeutic results. A complication of the technique is that the tourniquet can cause headache and nerve compression.

The shortfall of the scalp tourniquet technique led to exploration of new methods for preventing and treating alopecia. In the scalp cooling technique, the uniform cooling of the scalp causes vasoconstriction and decreases the metabolic rate of scalp blood vessels. A number of different cooling means have been explored for accomplishing the uniform cooling, including helmets attached to a room air conditioner, frozen polyethylene gel packs, frozen cryogel bags and plastic bags containing ice. The discomfort caused by these methods can be oppressive. In addition, the cumbersome nature of the cooling means amplifies the discomfort, rendering scalp cooling a relatively unfavorable method of treatment. Perlin, E., et al, "Protection from Chemotherapy-Induced Alopecia," *Medical and Pediatric Oncology* 19:129-130 (1991).

Until the early 1990s, the development of new chemical therapies had been restrained by the lack of a suitable and reproducible experimental model. In 1990, Hussein et al. reported testing a biologic response modifier prepared from the bacterium *Serratia marcescens*, Imuvert, in a newborn rat model. The newborn rats were administered cytosine arabinoside or adriamycin chemotherapies. The data collected demonstrated that the animals were protected from the chemotherapy induced-alopecia with a high degree of reproducibility. Hussein, et al., *Science* 249:1564-1566 (1990). Subsequent studies demonstrated similar protection from vidarabine (ARA-C) induced alopecia by using recombinant interleukin-1 (IL-1) beta in the same model. Jimenez, et al., FASEB J. (1991).

Recently, a promising new treatment has been tested in the newborn rat model by Jimenez et al. As reported in U.S. Patent No. 5,486,509, Jimenez et al. use vitamin D_3 compounds, in particular 1,25-dihydroxyvitamin D_3 [or $1,25(OH)_2D_3$], for preventing and treating alopecia induced by chemotherapeutic agents. Topical pretreatment of young rats using $1,25(OH)_2D_3$ for six days protected the animals from cytoxan (CTX), etoposide (VP-16), adriamycin (ADM),

cytoxan in combination with adriamycin (CTX + ADM), and paclitaxel induced alopecia (Jimenez et al., 1991; Jimenez et al., 1992a; Jimenez et al., 1992d). However, vitamin D_3 compounds, including $1,25(OH_2)D_3$, have been known to exhibit hypercalcemic effects in a clinical setting. Accordingly, concern exists that vitamin D_3 compounds may not be suitable for preventative therapy or as a therapeutic agent for the treatment of chemotherapy-induced alopecia in the clinical setting.

Therefore, there remains a need for novel methods of preventing and treating chemotherapy-induced alopecia, and in particular, for accomplishing such prevention and treatment in the clinical setting. A preferred method will involve a compound demonstrating less calcemic effect while maintaining the beneficial quality of preventing and treating hair loss attributed to chemotherapy.

Summary of the Invention

The invention provides a method of preventing or treating chemotherapy-induced alopecia. In the claimed method, a vitamin D_2 compound is administered to a patient affected with an alopecic condition caused by a chemotherapeutic agent. The compounds suitable for the method exhibit less calcemic effect than vitamin D_3 derivatives. Compounds for use in the method have demonstrated *in vivo* protection against alopecia induced by the administration of a chemotherapeutic agent or a combination of chemotherapeutic agents.

Brief Description of the Drawings

FIG. 1 is a photograph of 6 rats from Experiment III. All rats received 1.5 mg/kg of etoposide for 3 consecutive days. The 3 rats on the left received placebo lotion administered topically on day 5 after birth and continuing through day 10. The 3 rats on right received 1α,25-dihydroxy-19-nor-vitamin D_2 applied topically (0.1 mL per square centimeter of surface area treated) beginning on day 5 after birth and continuing through day 10.

FIG. 2 is a photograph of 6 rats from Experiment IV. All rats received 35 mg/kg of cytoxan for 1 day. The 3 rats on the left received placebo lotion administered topically on day 5 after birth and continuing through day 10. The 3 rats on the right received 1α,25-dihydroxy-19-

nor-vitamin D_2 applied topically (0.25 mL per square centimeter of surface area treated) beginning on day 5 after birth and continuing through day 10.

FIG. 3 is a photograph of 6 rats from Experiment V. All rats received 35 mg/kg of cytoxan for 1 day followed by 2.5 mg/kg for 3 days. The 3 rats on the left received placebo lotion administered topically on day 5 after birth and continuing through day 10. The 3 rats on the right received 1α,25-dihydroxy-19-nor-vitamin D_2 applied topically (0.25 mL per square centimeter of surface area treated) beginning on day 5 after birth and continuing through day 10.

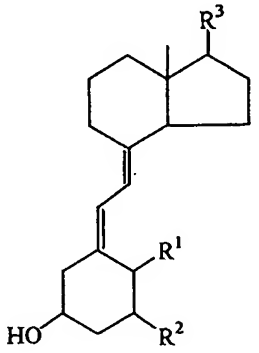
FIG. 4 shows the cross-section for a skin biopsy taken from the control rats used for obtaining blood serum. All control rats received placebo lotion administered topically on day 5 after birth and continuing through day 10. The lotion was applied daily over the head, neck and back. After application of the lotion, the rats were kept individually separated for a period of 3 hours. The area having lotion was carefully washed at the end of three hours. Skin biopsies were taken from the rats and placed in formaldehyde. The biopsies were processed and stained with H&E. A cross-section of a skin biopsy from a control rat is shown.

FIG. 5 shows the cross-section for a skin biopsy taken from the treated rats used for obtaining blood serum. All treated rats received 1α,25-dihydroxy-19-nor-vitamin D_2 administered topically as a lotion on day 5 after birth and continuing through day 10. The lotion was applied daily over the head, neck and back. After treatment with the lotion, the rats were kept individually separated for a period of 3 hours. The treated area was carefully washed at the end of three hours. Skin biopsies were taken from the rats and placed in formaldehyde. The biopsies were processed and stained with H&E. A cross-section of a skin biopsy from a treated rat is shown.

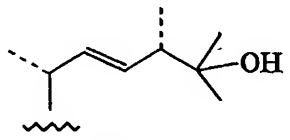
Detailed Description of the Invention

The term "vitamin D_2 compound" as used throughout the specification and claims shall refer to any vitamin D_2 compound, an analog, derivative, or active metabolite thereof.

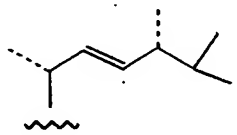
The method of inhibiting chemotherapy-induced alopecia comprises administering to a host, treated with at least one chemotherapeutic agent, an effective amount of a vitamin D_2 compound. The compounds useful for the claimed invention have the structural formula:



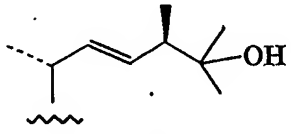
wherein R^1 represents hydrogen or a methylene group; R^2 is hydrogen or hydroxy; and R^3 represents an unsaturated, aliphatic alkyl group optionally substituted with methyl or hydroxy, provided that R^1 and R^2 are not both hydrogen. The preferred substituents for R^1 and R^2 are methylene and hydroxy, respectively. The group defined as R^3 represents a side chain common to vitamin D_2 compounds. Examples of suitable side chains for the compounds include, but are not limited to:



(a)

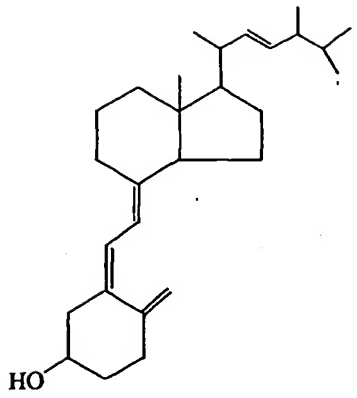


(b) and

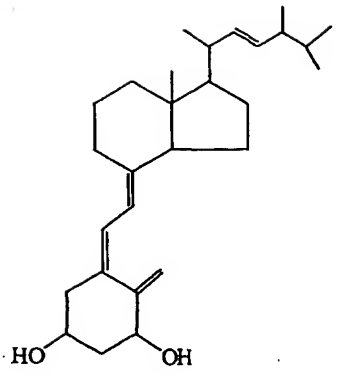


(c)

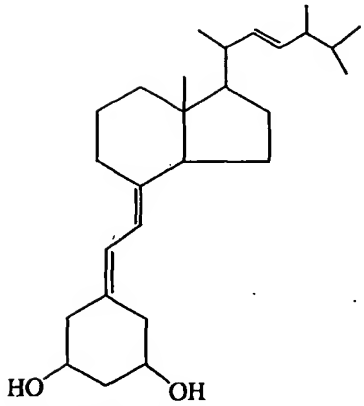
It is preferred that the side chain is a group represented by (a) or (c) above. Compounds contemplated as part of the invention include, but are not limited to:



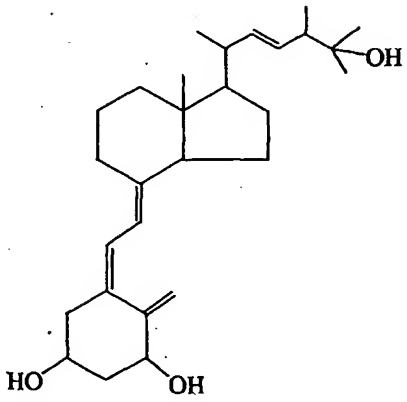
vitamin D_2,



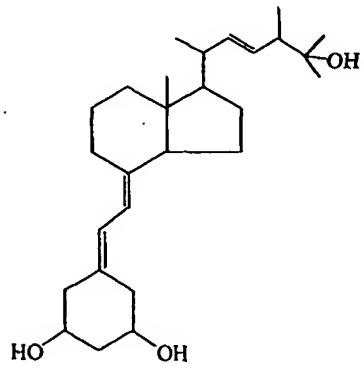
1α-hydroxyvitamin D_2,



1α-hydroxy-19-nor-vitamin D_2,



1α, 25-dihydroxyvitamin D_2, and



1α,25-dihydroxy-19-nor-vitamin D_2.

The preferred compound for the method of the invention is 1α,25-dihydroxy-19-nor-vitamin D_2, also referred to herein as paricalcitol or 19-nor D_2. A preparation of paricalcitol is commercially

available as ZEMPLAR® from Abbott Laboratories (Abbott Park, IL U.S.A.) or can be prepared by methods previously described in the literature. The preparation of vitamin D_2 compounds useful in the invention, the reagents, conditions, and procedures suitable for the preparation of vitamin D_2 compounds has been described in the literature in at least U.S. Patent Nos. 4,195,027; 4,260,549; 4,448,721; 4,769,181; 5,030,772; 5,237,110; 5,321,018; 5,342,975, and by Paaren et al. in *J. Org. Chem.* 48, 3819 (1983), which are incorporated by reference.

Vitamin D_2 compounds exhibit unexpected activity in reducing, preventing or treating the condition of hair loss induced by a chemotherapeutic agent. The compounds have demonstrated activity by inhibiting patterns of chemotherapy-induced hair loss *in vivo* in a newborn rat model. The pattern of hair growth in the newborn rats is comparable with the growth pattern of the hair follicles in the human head, i.e. approximately 90% of the scalp hairs are in the active growing phase, anagen, and the remaining 10% are in the resting or shedding phase, telogen. The model is effective for predicting the prevention or treatment of alopecia *in vivo* in a patient undergoing treatment with chemotherapeutic agents. See, for example, Jimenez, J.J. et al. *FASEB J.* 1992b: 6:911-13; Jimenez, J.J., et al. *Cancer Research* 1992c, 52:413-15; and Jimenez, J.J. et al. *Am. J. Med. Sci.* 1995, 310:43-47.

The compounds can be administered to a host, or patient, treated with at least one chemotherapeutic agent that induces alopecia. Generally, the host or patient is a mammal, including humans and animals. The host or patient is the recipient of therapeutic treatment with either a chemotherapeutic agent or a combination of chemotherapeutic agents. Typically, a suitable combination therapy will have two or three chemotherapeutic agents. The chemotherapeutic agents can be either cell cycle specific or non-cell cycle specific. The compounds have demonstrated preventative effect in newborn rat host treated with cytoxan, a combination of adriamycin and cytoxan, and etoposide. The compounds can have effect in reducing, preventing or treating alopecia induced by other chemotherapeutic agents as well, including adriamycin alone, cytosine arabinoside, doxorubicin, 5-fluorouracil, and paclitaxel.

An effective amount of a vitamin D_2 compound can be determined in accordance with the guidelines of the health practitioner administering the medication considering the needs of the patient, including the severity of the condition to be treated, and the response or conditions of the

subject. These methods are well-understood in the art; however, for the convenience of the reader, it is suggested that a suitable dosage amount of the vitamin D_2 compound is from about 0.05 microgram to about 0.2 microgram of compound per square centimeter of surface area treated. Preferably, the dosage amount is about 0.125 micrograms of compound per square centimeter of surface area treated.

The compounds can be formulated as solutions in innocuous solvents, or as emulsions, suspensions or dispersions in a pharmaceutically acceptable solvent or carrier. To achieve the full benefit of the invention, the compounds can be formulated into solutions, lotions, creams, ointments, gel, emulsions or other similar vehicles for topical application. It is conceivable that the compound can be formulated into other dosage forms, including oral, intraperitoneal, or subcutaneous dosage forms, for example a tablet, capsule, emulsion, microemulsion, suspension or injectable solution. Any such formulation may also contain a combination of one or more solvents or carriers. The combination can optionally contain other pharmaceutically acceptable and non-toxic excipients, such as stabilizers, anti-oxidants, binders, coloring agents or emulsifying agents. The topical dosage forms can also include olfactory-enhancing agents. Oral dosage forms can optionally incorporate taste-modifying agents to pleasantly improve the overall desirability of the product.

A preferred topical formulation comprises the vitamin D_2 compound in a pharmaceutically acceptable aqueous medium or pharmaceutically acceptable hydroalcoholic preparation. As used herein, an aqueous medium means a water-based solution without organic solvent and a hydroalcoholic preparation means a water-based solution comprising one or more alcohols. The vitamin D_2 compound can be dissolved in an organic solvent, preferably alcohol. The alcohol and one or more pharmaceutically acceptable adjuvants are blended to provide a homogenous formulation. Suitable alcohols for the invention include methanol, ethanol, isopropyl alcohol, and the like. The preferred alcohol is isopropyl alcohol. The adjuvant that can be used in the formulation of the invention is preferably a cosolvent, for example propylene glycol, low molecular weight polyethylene glycol, and the like, alone or in combination with water. It is more preferred that the cosolvent is propylene glycol, more preferably in combination with water.

Additional non-therapeutic ingredients can also be incorporated into the formulation. The non-active ingredients can include excipients, adjuvants or additives, including suspending agents or gelling agents such as bentonite and magnesium aluminum silicate; thickening agents such as agar, cellulose, tragacanth, pectin, sodium alginate, methylcellulose, carboxymethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, sodium carboxymethylcellulose, and a combination of carbomer and carboxypolymethylene; humectants such as glycerin and sorbitol; preservatives such as phenylethyl alcohol or hydroxy benzoate esters; and antioxidants such as tocopherol or alkyl gallates.

A preferred hydroalcoholic preparation comprises from about 2 micrograms per milliliter to about 40 micrograms per milliliter of a vitamin D_2 compound, from about 25% to about 75% volume/volume of alcohol; and about 75% to about 25% volume/volume of a pharmaceutically acceptable adjuvant in water. An example of a preferred formulation suitable for the method of the invention comprises 20 micrograms of 1α,25-dihydroxy-19-nor-vitamin D_2 per milliliter of solution, wherein the solution comprises from about 51% volume/volume of isopropyl alcohol; about 4% volume/volume of propylene glycol; about 1% weight /volume hydroxypropylcellulose; and sufficient water to yield the desired volume.

The preferred formulation is prepared by combining the isopropanol, propylene glycol and approximately forty percent of the final volume of distilled water in a suitably sized container or flask. The hydroxypropylcellulose is added to the above solution and stirred for a sufficient period of time to completely disperse the hydroxyproplycellulose. Paricalcitol is added and stirred until dissolved. The resulting mixture is adjusted to final volume with distilled water.

The preferred dosing regimen is carried out in a manner to assure the delivery the compound to the affected area, for example, the hair follicles. Particularly in the case of chemotherapy-induced alopecia, the affected area more specifically refers to the hair follicles in the scalp.

The invention can be better understood in connection with the following Examples, which are intended to illustrate the invention and should not be construed as limiting the scope of the invention as defined in the appended claims.

EXAMPLES

Preparation of 1α,25-Dihydroxy-19-nor-Vitamin D_2 Formulation

The 1α,25-dihydroxy-19-nor-vitamin D_2 formulation used in the following experiments was prepared from the commercially available ZEMPLAR® solution. For the following experiments, the concentration of paricalcitol in ZEMPLAR was increased from 2 micrograms per milliliter to 8 micrograms per milliliter.

Biological Assessment of 1α,25-Dihydroxy-19-nor-Vitamin D_2

The following experimental details relate to Examples I-IV set forth below.

Sprague Dawley rats were purchased from Charles River Laboratories (Wilmington, Massachusetts, U.S.A.). Rats were fed and housed according to NIH guidelines. Daily weight gains were individually recorded. Cytoxan and adriamycin were obtained from Adria Laboratories (Columbus, Ohio, U.S.A.). Etoposide was obtained from Bristol Laboratories (Princeton, New Jersey, U.S.A.).

A solution of 19-nor-D_2 and a placebo were applied to newborn rats daily starting on day 5 after birth and continuing through day 10 after birth. The solution was applied topically over the head, neck and back. After treatment with solution, animals were then kept individually separated for a period of 3 hours, following which the treated area was carefully washed. Daily weights were recorded to determine systemic toxicity.

Chemotherapy was given intraperitoneally (I.P.) and began at 11 days of age. Cytoxan (CTX, 35 mg/kg) was given for one day only. A combination of cytoxan and adriamycin (CTX + ADM) was administered as follows: CTX (25 mg/kg) for 1 day and adriamycin (ADM, 2.5 mg/kg) for 3 days. Etoposide (VP-16, 1.5 mg/kg) was given for 3 consecutive days.

Ten days after chemotherapy, rats were evaluated for hair loss and photographs were taken. The following scale was used to rank alopecia: no detectable alopecia was 0; less than 50% hair loss was 1; more than 50% hair loss was 2; and total absence of hair was 3.

Examples I - III

Determination of Minimal Effective Dose to Protect Against Alopecia

The first three sets of experiments were conducted to screen for protection and to determine the minimal effective dose of 19-nor-D_2. For this purpose the chemotherapy used was etoposide. For each experiment, six, 5-day-old rats were randomized in two groups of three each. Group 1 received 19-nor-D_2. Group 2, received placebo and served as control.

Example I

Group 1 received 0.1 mL of 19-nor-D_2 and group 2 received placebo solution. In group 1, there was significant evidence of protection. One rat was completely protected from developing alopecia, scale 0, one rat ranked as a 1 on the scale, and the other as a 2. All the rats in group 2 became totally alopecic, scale 3. There was no evidence of skin irritation, or of systemic toxicity, as indicated by the change in body weight, Table 1.

Table 1

Number of animals at each alopecia level				
Alopecia level	**0**	**1**	**2**	**3**
Control	0	0	0	3
19-nor-D_2 (0.1 mL)	1	1	1	0
Weights (grams)				
	Control		**19-nor-D_2**	
Day 5 (after birth)	12 ± 0.5		12.5 ± 0.5	
Day 11	21 ± 1.0		22 ± 1.0	
Day 15	25 ± 1.5		26 ± 1.0	
Day 18	31 ± 1.5		31.5 ± 1.5	

Example II

The dose of 19-nor-D_2 was increased to 0.2 mL. One rat was completely protected from developing alopecia, scale 0, two rats ranked as a 1 on the scale, and the other as a 2. No rats in group 1 became totally alopecic, as indicated by having no animals ranked as scale 3. All the rats in group 2 became totally alopecic. The results are reported in Table 2.

Table 2

Number of animals at each alopecia level				
Alopecia level	**0**	**1**	**2**	**3**
Control	0	0	0	3
19-nor-D_2 (0.2 mL)	1	2	1	0
Weights (grams)				
	Control	**19-nor-D_2**		
Day 5 (after birth)	13 ± 0.5	12.5 ± 0.5		
Day 11	22 ± 1.0	22 ± 1.0		
Day 15	27 ± 1.5	26 ± 1.5		
Day 18	33 ± 1.5	32.5 ± 1.5		

As shown in Table 2, the protection of the rats from alopecia was improved. No evidence of toxicity was noted, as indicated by the recorded weights.

Example III

The dose of 19-nor-D_2 was increased to 0.25 mL. Results of the testing are reported in Table 3.

Table 3

Number of animals at each alopecia level				
Alopecia level	**0**	**1**	**2**	**3**
Control	0	0	0	3
19-nor-D_2 (0.25 mL)	3	0	0	0
Weights (grams)				
	Control	**19-nor-D_2**		
Day 5 (after birth)	11.5 ± 0.5	12 ± 0.5		
Day 11	20 ± 1.0	21 ± 1.0		
Day 15	24 ± 1.0	24.5 ± 1.5		
Day 18	30 ± 1.5	32 ± 1.5		

As illustrated in Table 3, the treated rats were completely protected from developing alopecia. At this increased dose, no evidence of local or systemic toxicity was noted. A picture of the control group and the treated group is shown in FIG. 1.

Examples IV – V

Demonstration of Protection against Chemotherapy-induced Alopecia

The procedure from example III, above, was repeated but substituting the procedures for carrying out the CTX (Example IV) and CTX+ADM (Example V) chemotherapies, respectively. The 19-nor-D_2 was applied at a volume of 0.25 mL.

Example IV

Complete protection from CTX-induced alopecia was demonstrated without evidence of local or systemic toxicity as reported below in Table 4. A picture of the control group and the treated group is shown in FIG. 2.

Table 4

Number of animals at each alopecia level				
Alopecia level	**0**	**1**	**2**	**3**
Control	0	0	0	3
19-nor-D_2 (0.25 mL)	3	0	0	0
Weights (grams)				
	Control		**19-nor-D_2**	
Day 5 (after birth)	12.5 ± 0.5		12 ± 0.5	
Day 11	21 ± 1.0		21.5 ± 1.0	
Day 15	25.5 ± 1.0		26.5 ± 1.5	
Day 18	31.5 ± 1.5		32 ± 1.5	

Example V

Complete protection from the CTX-ADM combination therapy was obtained without evidence of local or systemic toxicity as shown in Table 5. A picture of the control group and the treated rats is shown in FIG. 3.

Table 5

Number of animals at each alopecia level				
Alopecia level	**0**	**1**	**2**	**3**
Control	0	0	0	3
19-nor-D_2 (0.25 mL)	3	0	0	0
Weights (grams)				
	Control		**19-nor-D_2**	
Day 5 (after birth)	11 ± 0.5		12 ± 0.5	
Day 11	19 ± 1.0		20 ± 1.0	
Day 15	23 ± 1.0		24 ± 1.0	
Day 18	28 ± 1.5		28.5 ± 1.5	

Example VI

Effect of 19-nor-vitamin D_2 on Calcium and PTH levels

Six 5-day-old rats were randomized into two groups of 3 each. Group 1, received 0.25 mL of 19-nor-D_2 topically starting on day 5 after birth and continued through day 10 after birth. Group 2 received placebo similarly.

To determine levels of calcium and pituitary thyroid hormone (PTH), the 19-nor-D_2 applied daily starting on day 5 after birth and continued through day 10 after birth. The solution was applied topically over the head, neck and back. After treatment with solution, animals were then kept separated for a period of 3 hours, following which the treated area was carefully washed. Daily weights were recorded to determine systemic toxicity. Eleven days after birth, the rats were exanguinated and the blood collected. After centrifugation, the serum collected was frozen at -70 °C. Subsequently the aliquoted serum samples were assayed for PTH and calcium levels. The results are reported in Table 6, below.

Table 6

	Calcium (mg/dl)	PTH (pg/dl)
Control Group		
Rat 1	9.4	58.81
Rat 2	9.2	66.53
Rat 3	6.7	60.24
Treated with 19-nor-D_2		
Rat 1	6.1	73.30
Rat 2	9.2	33.35
Rat 3	9.2	46.40

As shown in Table 6, there was no significant difference in calcium levels between the control and 19-nor-D_2 treated groups. Two rats in the 19-nor-D_2 group had lower levels of PTH than the control group. One rat had a higher level.

Example VII

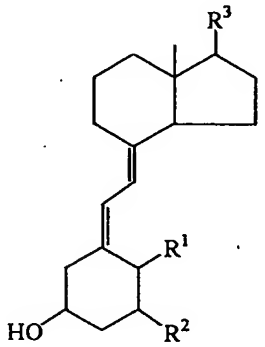
Histological Assessment of Hair Follicles

Skin biopsies where taken from control and 19-nor-D_2 treated rats (used for obtaining serum). The biopsies were placed in formaldehyde and later processed and stained with H&E (hematoxylin and eosin) stain. The morphological assessment revealed no significant differences between the control and 19-nor-D_2 hair follicles, Fig. 4 (control) and Fig. 5 (19-nor-D_2). In addition there was no evidence of local toxicity.

WHAT IS CLAIMED IS:

1. A method of preventing or treating chemotherapy-induced alopecia in a mammal, comprising administering to said mammal an effective amount of vitamin D_2 compound.

2. The method according to Claim 1, comprising administering a compound having the formula:



or an analog, derivative or active metabolite thereof, wherein R^1 represents hydrogen or a methylene group; R^2 is hydrogen or hydroxy; and R^3 represents an unsaturated, aliphatic alkyl group optionally substituted with methyl or hydroxy, provided that R^1 and R^2 are not both hydrogen.

3. The method according to Claim 2, wherein the compund is vitamin D_2, 1α-hydroxyvitamin D_2, 1α-hydroxy-19-nor-vitamin D_2, 1α,25-dihydroxy-19-nor-vitamin D_2 and 1α,25-dihydroxyvitamin D_2.

4. The method according to Claim 3, wherein the compound is 1α,25-dihydroxy-19-nor-vitamin D_2.

5. The method according to Claim 1, wherein the mammal receives treatment with at least one chemotherapeutic agent which induces alopecia.

6. The method according to Claim 5, wherein the chemotherapeutic agent is cytarabine arabinoside, cytoxan, adriamycin, etoposide, doxorubicin, paclitaxel, 5-fluoro-uracil, or a combination of these agents.

7. The method according to Claim 6, wherein the chemotherapeutic agent is cytoxan, adriamycin, etoposide, or a combination of cytoxan and adriamycin.

8. The method according to Claim 1, wherein the vitamin D_2 compound is administered topically.

9. The method according to Claim 8, wherein the vitamin D_2 compound is administered prior to treatment with one or more chemotherapeutic agents.

10. The method according to Claim 8, further comprising administering a formulation having from about 2 to about 40 micrograms per milliliter of vitamin D_2 compound in an aqueous medium, wherein said aqueous medium comprises from about 25 to about 75% (volume/volume) alcohol and from 75 to about 25 % (volume/volume) of a pharmaceutically acceptable aqueous medium.

11. The method according to claim 10, comprising 20 micrograms per millilter of 1α,25-dihydroxy-19-nor-vitamin D_2, 51% (volume/volume) isopropyl alcohol, 4% (volume/volume) propylene glycol; 1% (weight/volume) hydroxypropylcellulose; and sufficient water to yield the desired volume.

12. The method according to Claim 1, wherein the formulation comprises 8 micrograms of vitamin D_2 compound in 2 milliliters of aqueous medium, wherein said aqueous medium comprises about 20% volume/volume alcohol; about 30% volume/volume propylene glycol and about 50% volume/volume water.

13. The method according to Claim 12, wherein the vitamin D_2 compound is 1α,25-dihydroxy-19-nor-vitamin D_2.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/29980

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/59 A61P17/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 00079 A (UNIV MIAMI) 7 January 1993 (1993-01-07) page 3, line 5 - line 12; claims 1,9,21 page 6, line 30 - line 34	1,5,9
X	JIMENEZ JOAQUIN J ET AL: "Novel approaches to the prevention of chemotherapy-induced alopecia." EXPERIMENTAL BIOLOGY AND MEDICINE;NUTRIENTS IN CANCER PREVENTION AND, 1995, pages 333-345, XP001038605 1995 Humana Press Inc. Suite 808, 999 Riverview Drive, Totowa, New Jersey 07512, USA ISBN: 0-89603-318-X Abstract from biosis the whole document	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 January 2002	29/01/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Goss, I

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/29980

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOAQUIN J JIMENEZ ET AL: "Protection from chemotherapy-induced alopecia by 1,25-dihydroxyvitamin D3" CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, US, vol. 52, no. 18, 15 September 1992 (1992-09-15), pages 5123-5125, XP002095914 ISSN: 0008-5472 abstract page 5123, right-hand column, paragraph 2	1,5,7
Y	WO 99 16451 A (UNIV PITTSBURGH) 8 April 1999 (1999-04-08) page 7, line 15 - line 20	1,5,7
A	GB 2 139 627 A (WISCONSIN ALUMNI RES FOUND) 14 November 1984 (1984-11-14) page 1, line 5 - line 17	1
A	COTA J G ET AL: "JOURNAL OF ORGANIC CHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, US" JOURNAL OF ORGANIC CHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, US, vol. 26, no. 53, 1988, page 6094 XP002077584 ISSN: 0022-3263 indroduction	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 01/29980

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9300079 A	07-01-1993	AT 178206 T	15-04-1999
		AU 667675 B2	04-04-1996
		AU 2240592 A	25-01-1993
		CA 2112496 A1	07-01-1993
		CZ 9302892 A3	17-08-1994
		DE 69228814 D1	06-05-1999
		DE 69228814 T2	11-11-1999
		EP 0591378 A1	13-04-1994
		HU 66153 A2	28-09-1994
		IE 922088 A1	30-12-1992
		IL 102344 A	04-01-1998
		JP 6509072 T	13-10-1994
		NO 934844 A	24-02-1994
		PT 100638 A ,B	30-09-1993
		SK 149093 A3	10-08-1994
		WO 9300079 A1	07-01-1993
		US 5486509 A	23-01-1996
		US 6291443 B1	18-09-2001
		ZA 9204786 A	31-03-1993
		RU 2113850 C1	27-06-1998
WO 9916451 A	08-04-1999	US 6087350 A	11-07-2000
		AU 1359499 A	23-04-1999
		EP 1030670 A1	30-08-2000
		JP 2001517705 T	09-10-2001
		WO 9916451 A1	08-04-1999
GB 2139627 A	14-11-1984	AU 568549 B2	07-01-1988
		AU 3011584 A	04-12-1984
		BE 899612 A1	31-08-1984
		CH 665834 A5	15-06-1988
		DE 3448360 C2	02-10-1991
		DE 3448412 C2	12-12-1991
		DE 3490215 C2	25-07-1991
		DE 3490215 T	15-05-1985
		DK 8685 A	08-01-1985
		DK 183291 A	07-11-1991
		DK 184588 A	06-04-1988
		FR 2545824 A1	16-11-1984
		GB 2158442 A ,B	13-11-1985
		GB 2158443 A ,B	13-11-1985
		IE 58277 B	25-08-1993
		JP 1924384 C	25-04-1995
		JP 2288829 A	28-11-1990
		JP 6051624 B	06-07-1994
		JP 1676902 C	26-06-1992
		JP 2288873 A	28-11-1990
		JP 3039505 B	14-06-1991
		JP 1898026 C	23-01-1995
		JP 2288854 A	28-11-1990
		JP 6010188 B	09-02-1994
		JP 3064498 B	07-10-1991
		JP 60501261 T	08-08-1985
		MX 9203736 A1	01-09-1992
		NL 193245 C	02-04-1999
		NL 8420137 T	01-04-1985
		WO 8404527 A1	22-11-1984
		US 5036061 A	30-07-1991